(Adaptive) immune response and biofilms infections. Exemplified by chronic *Pseudomonas aeruginosa* lung infection.

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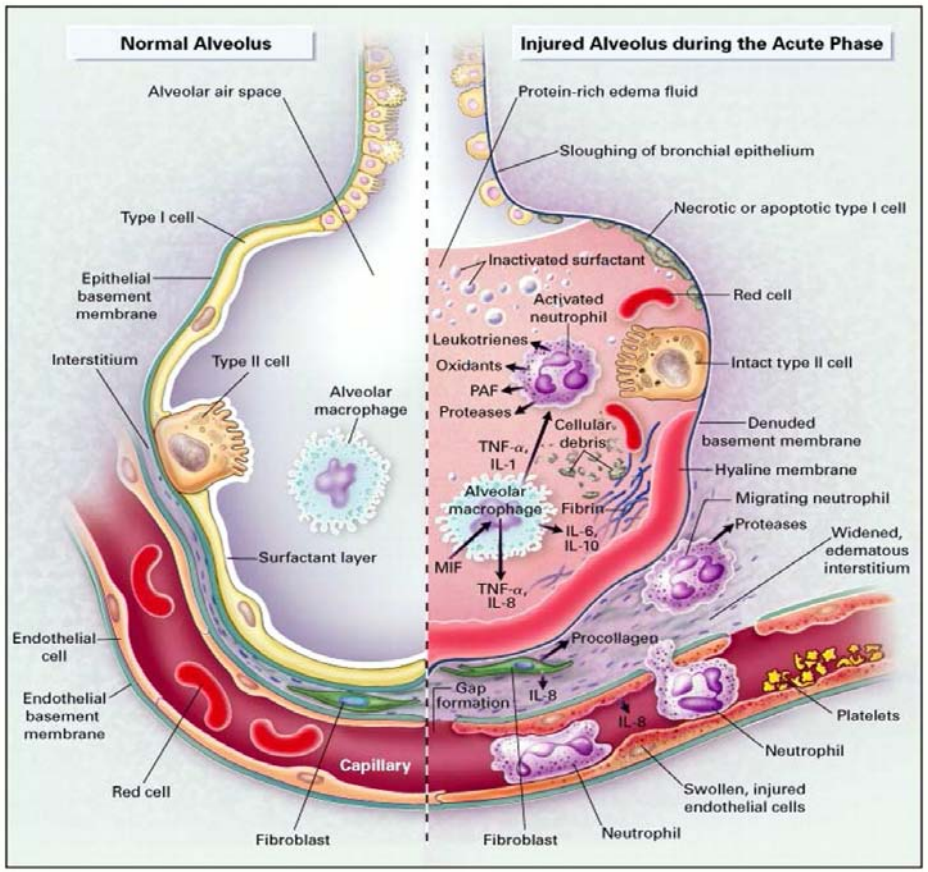
Introduction

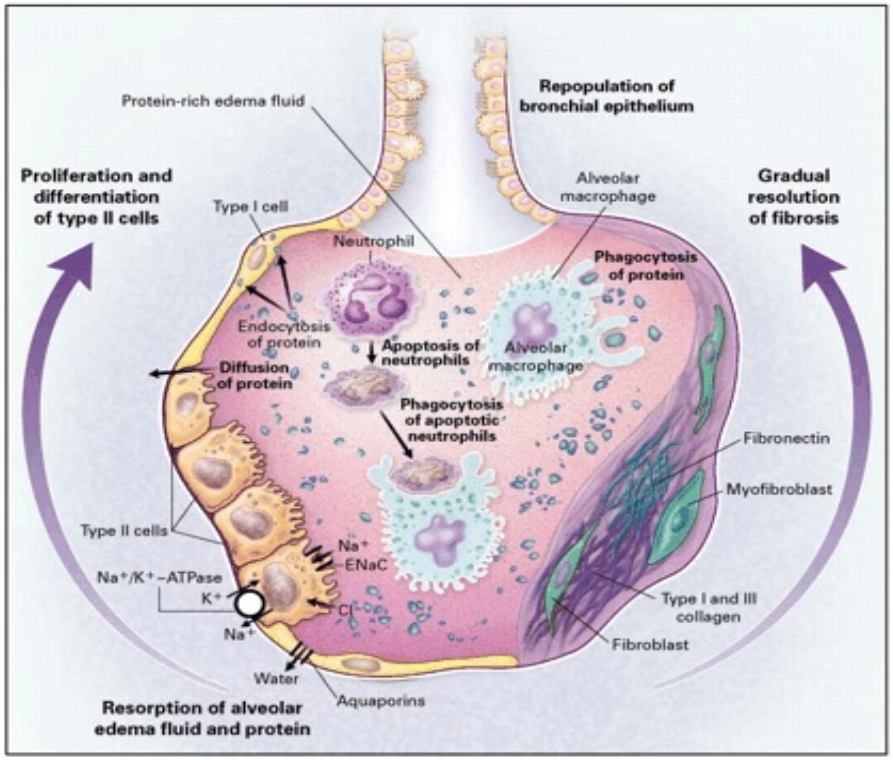
- Virulence factors and/or impaired host defense can result in biofilm infections.
- In cystic fibrosis reduced pericilliary fluid results in impaired mucus clearance.
- Recurrent and chronic lung biofilm infections.
- Activation of the immune responses uses the same mechanisms in biofilm infections as in non-biofilm infections.
- However, biofilm infections are resistant/tolerant to the effector mechanisms.
- Activation of both the innate and the adaptive immune responses without clearance of the pathogen -> Collateral tissue damage.

Acute lung infection

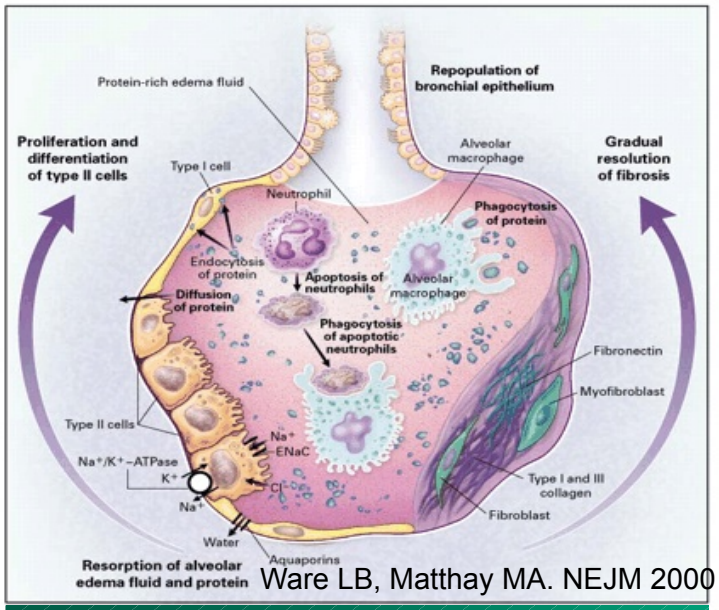


Ware LB, Matthay MA. NEJM 2000

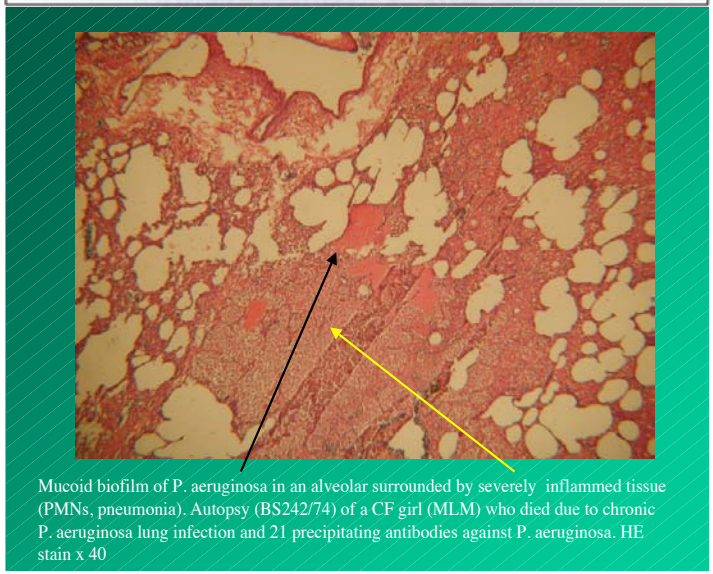
Resolution



Chronic biofilm infection in CF – no resolution.



Ware LB, Matthay MA. NEJM 2000

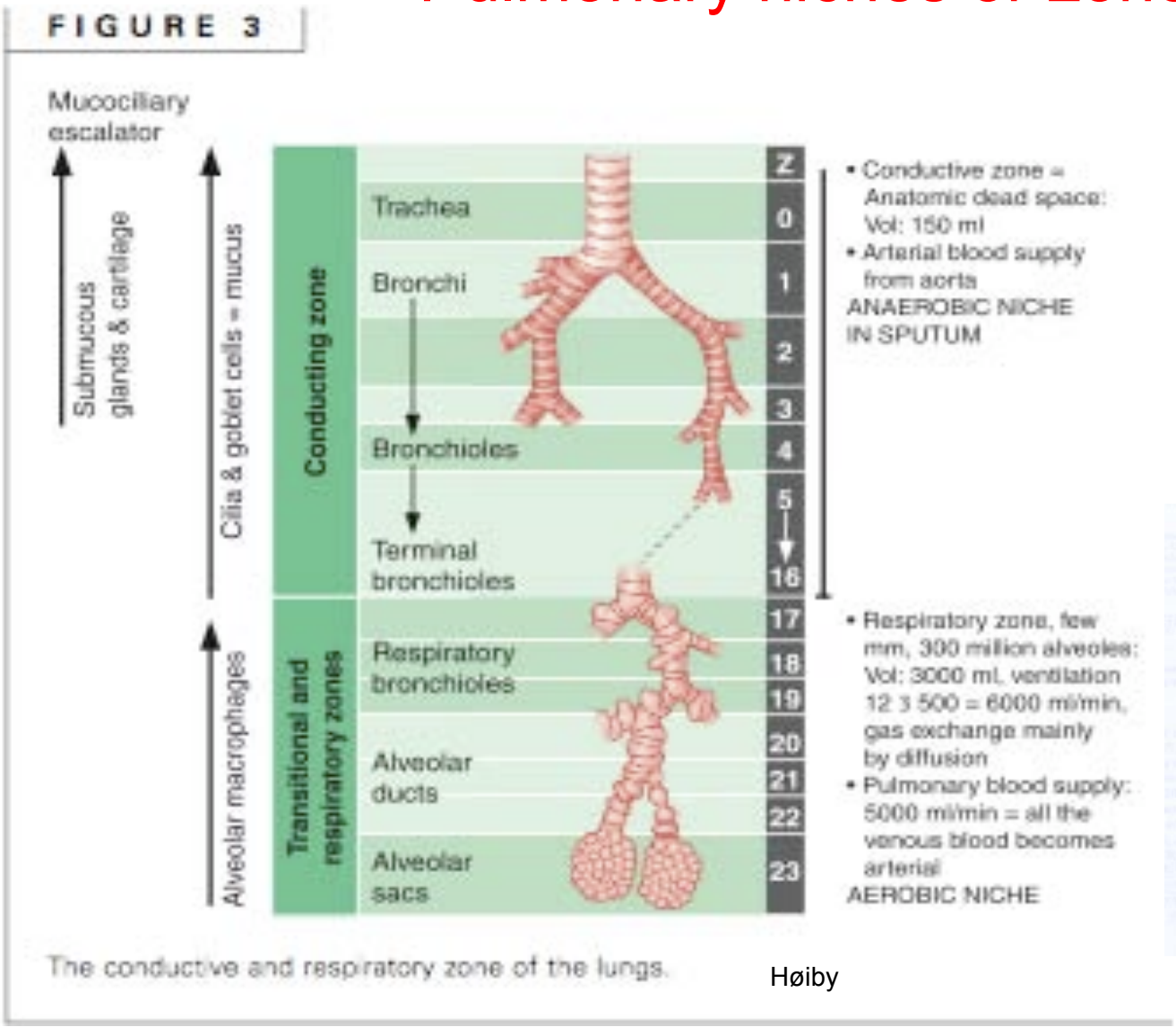


Mucoid biofilm of P. aeruginosa in an alveolar surrounded by severely inflammed tissue (PMNs, pneumonia). Autopsy (BS242/74) of a CF girl (MLM) who died due to chronic P. aeruginosa lung infection and 21 precipitating antibodies against P. aeruginosa. HE stain x 40

Courtesy Niels Høiby

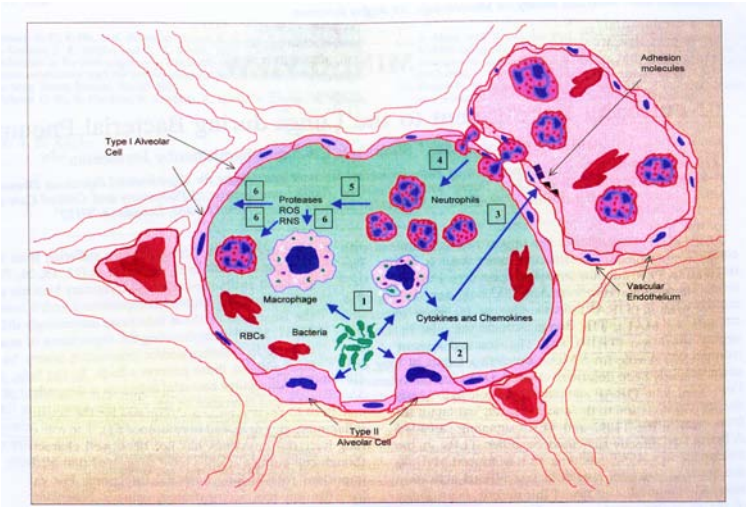
- **Adaptive immune response accelerates inflammation and contributes to pathogenesis:**
  - **Skewing of Th1/Th2 balance**
  - **Immunecomplex disease**
  - **Paradox persistent acute type inflammation (PMNs)**
  - **High number of necrotic PMNs**
  - **Progressive loss of lung function**
  - **Fibrosis**
- **The type of adaptive immune response influenced by the inflammatory response.**
- **Provides possible treatment targets.**

Pulmonary niches or zones



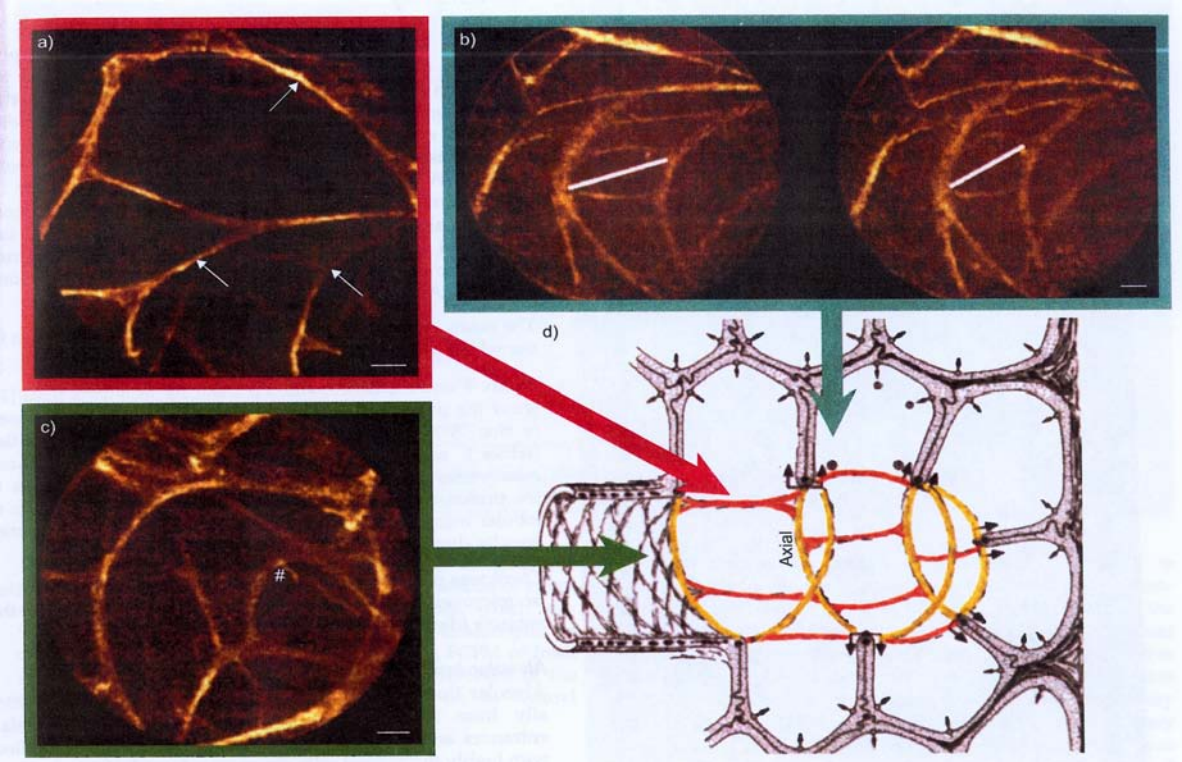
The conductive and respiratory zone of the lungs.

Høiby

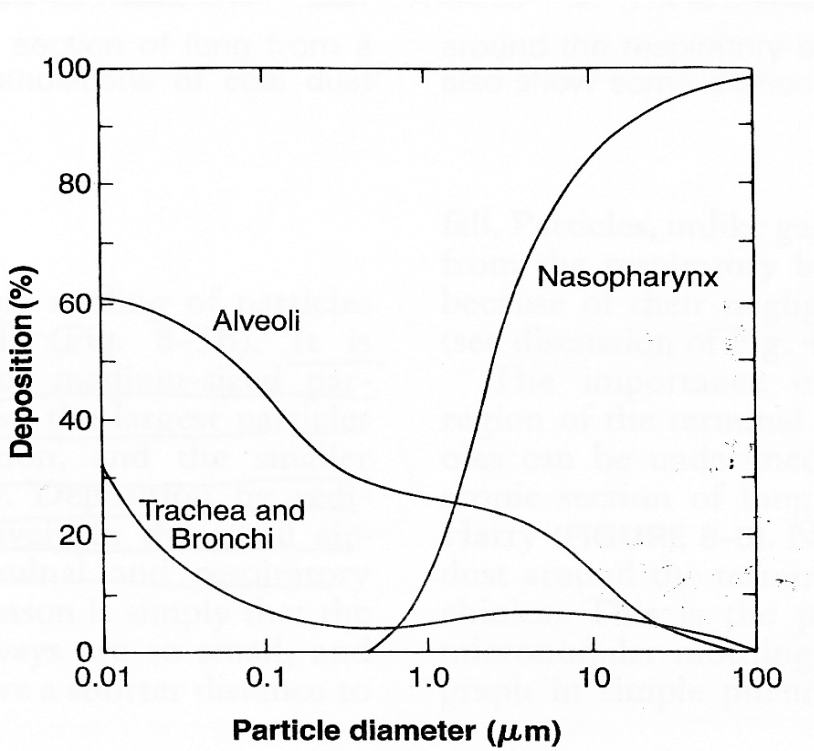


Craig A et al IAI 2009

Pulmonary dimensions



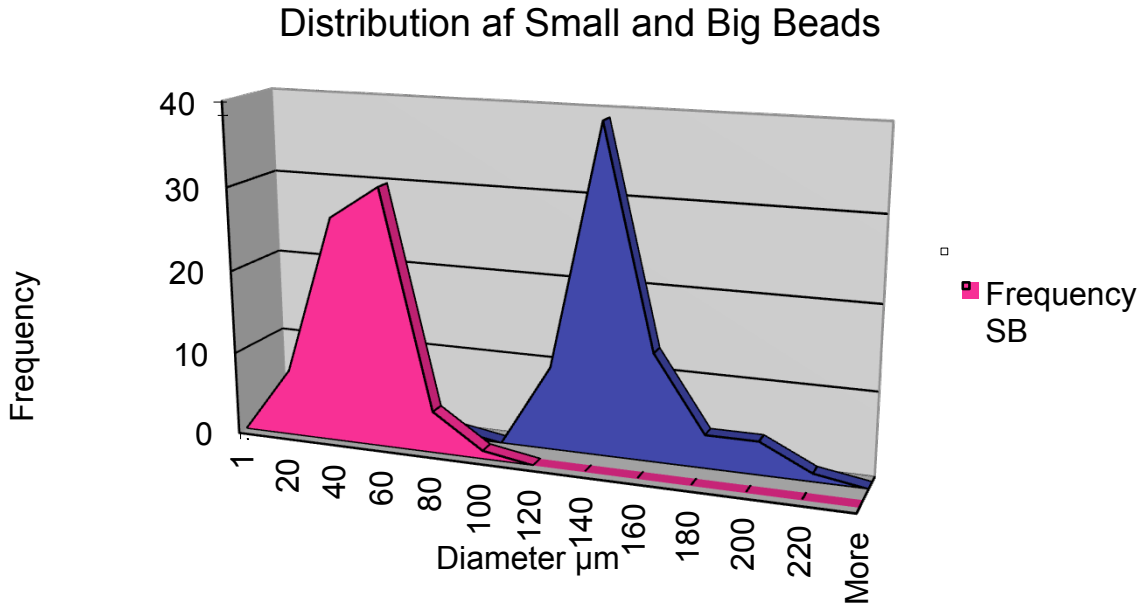
Thiberville L et al Eur Resp J 2009



West. Pulm phys and pathophys.
Lippincott Williams & Wilkins, 2001

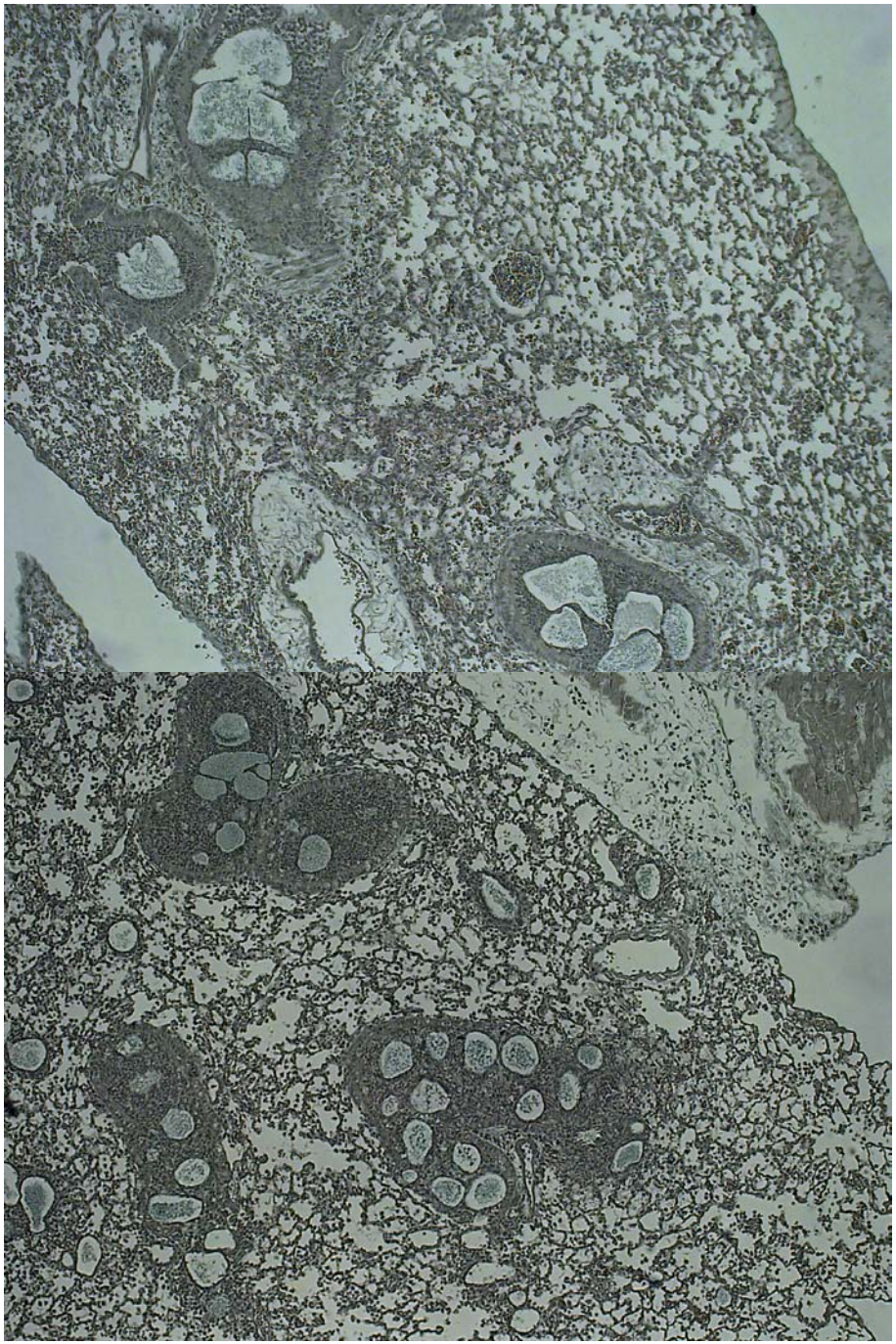
Localization matters.

Challenge of distinct lung niches.



Lars Christophersen et al. CEI 2012 in press

A

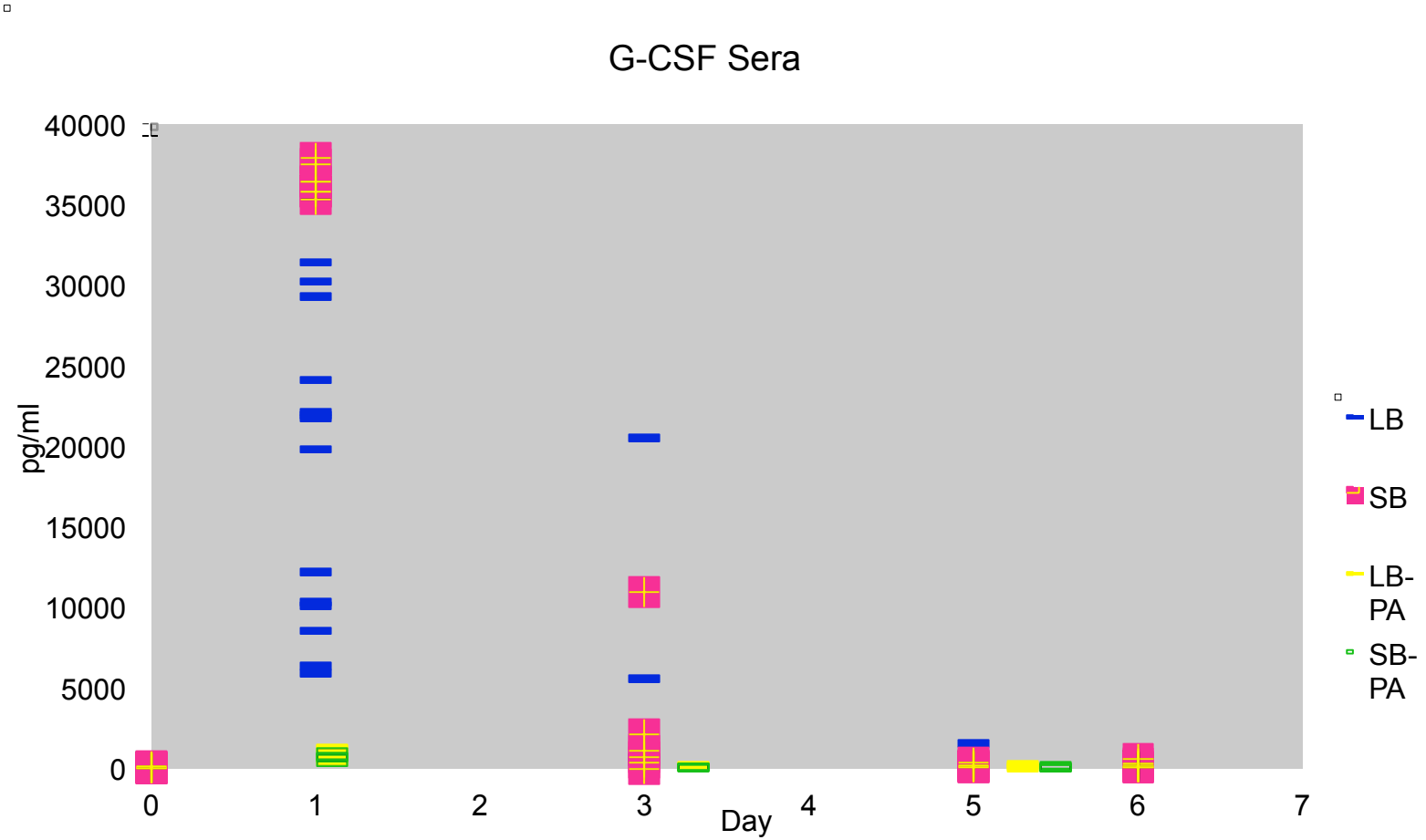


Large beads

B

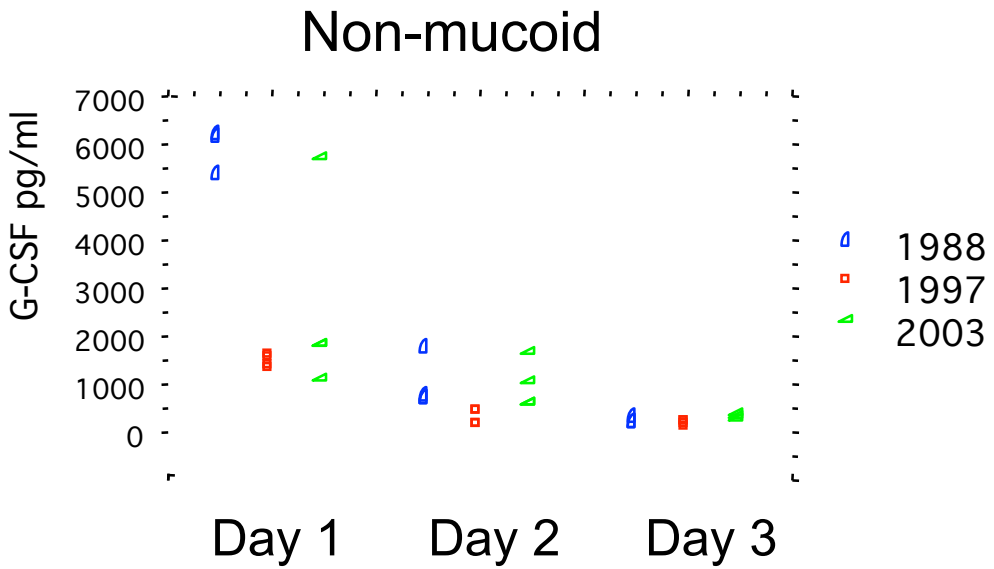
Small beads

L Christophersen et al. CEI 2012 in press

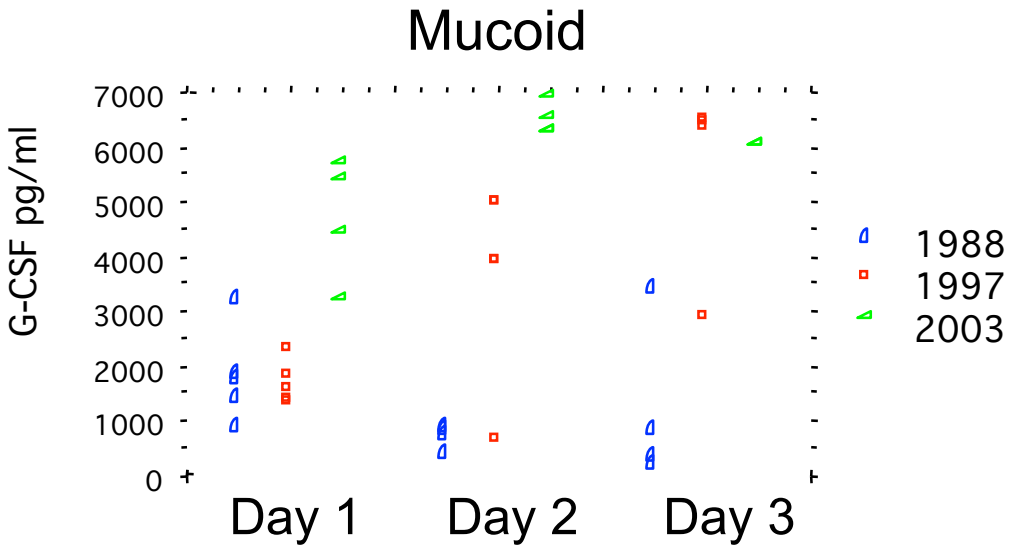


Lars Christophersen et al. CEI 2012 in press

Inflammatory respons
Non-mucoid versus mucoid.



G-CSF: PMN mobilizer from the bonemarrow



Moser and van Gennip et al 2009 and 2010

Traditional lymphocytes

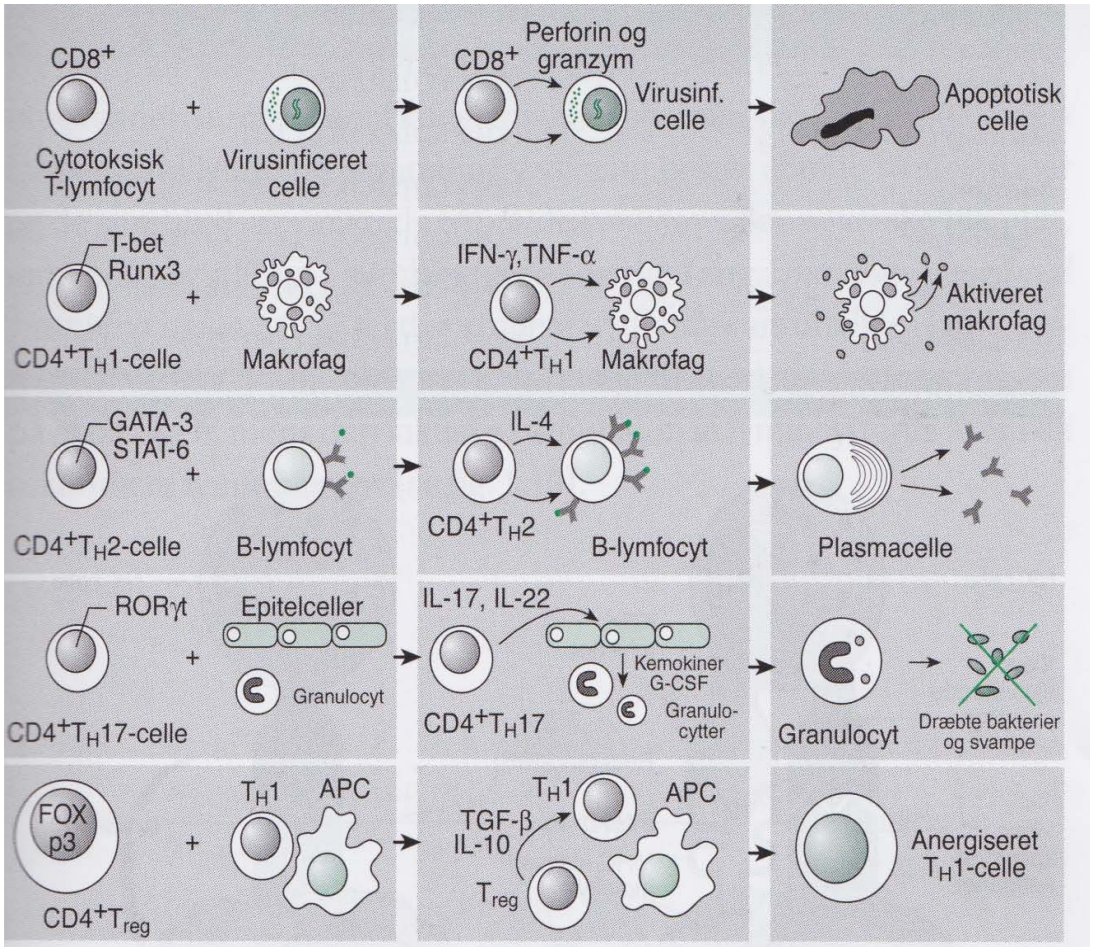
Cytotoxic T-cell

T-helper cell type1

T-helper cell type2

T-helper cell type17

T-regulatory cells



+ Induction of Tc

+ Mastcells

The new in class

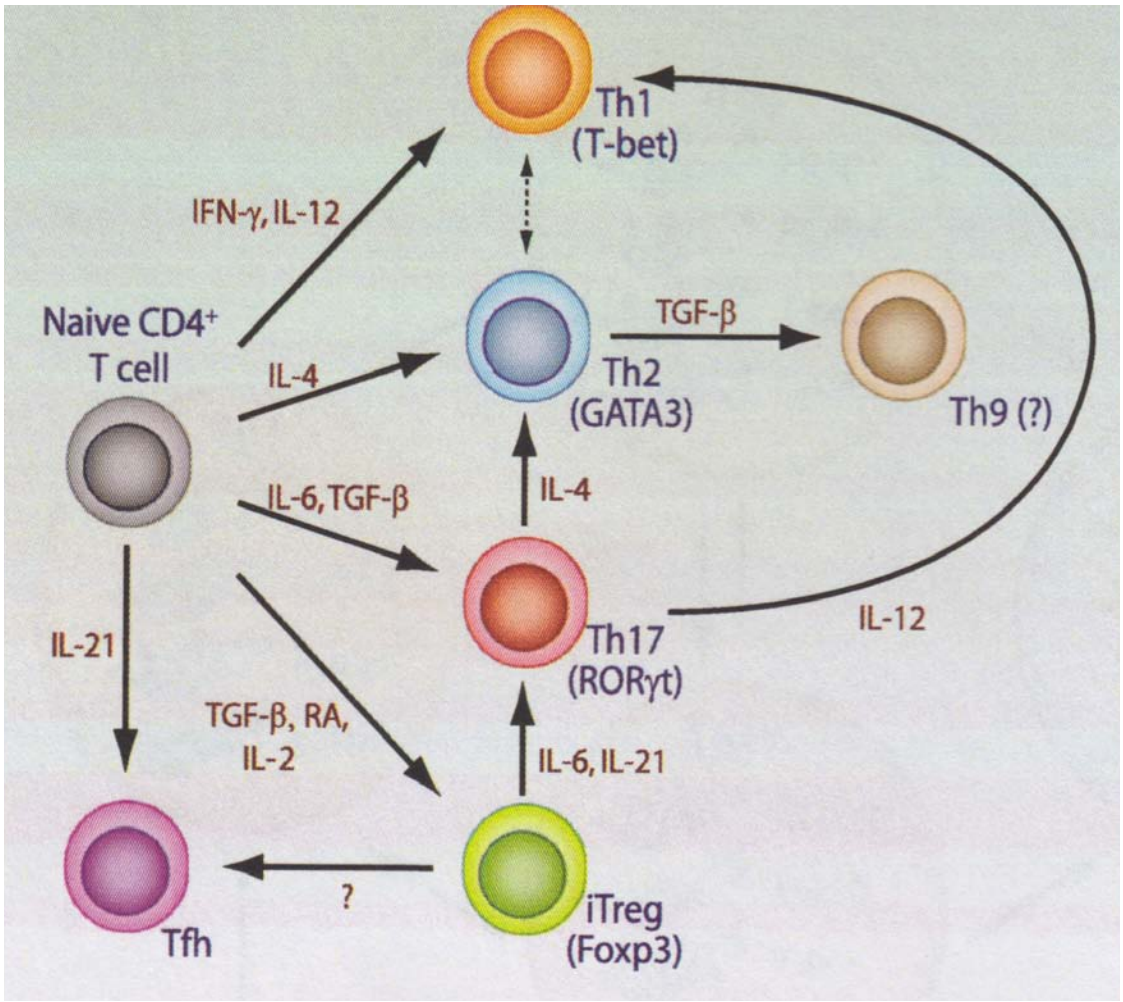
ST Lillevang and BK Møller. Immunologi 2009

Follicular Th cells, Tr1 and Th3 cells, NK and NKT cells, Central/peripheral memory cells.
α/β or γ/δ T lymphocytes.

$30x10^6$ cells/hour from one lymph node:
5% produced in the lymph node. 10% from afferent lymph.
85% recirculation through the high endothelial venules.

Recent sub divisions

Plasticity – the new buzz word! Emerging subsets!



Earlier believed to be fixed cell lineages.

Not the whole package each time.

Involvement of more subsets.

Seem to be relatively plastic:

Cytokine levels

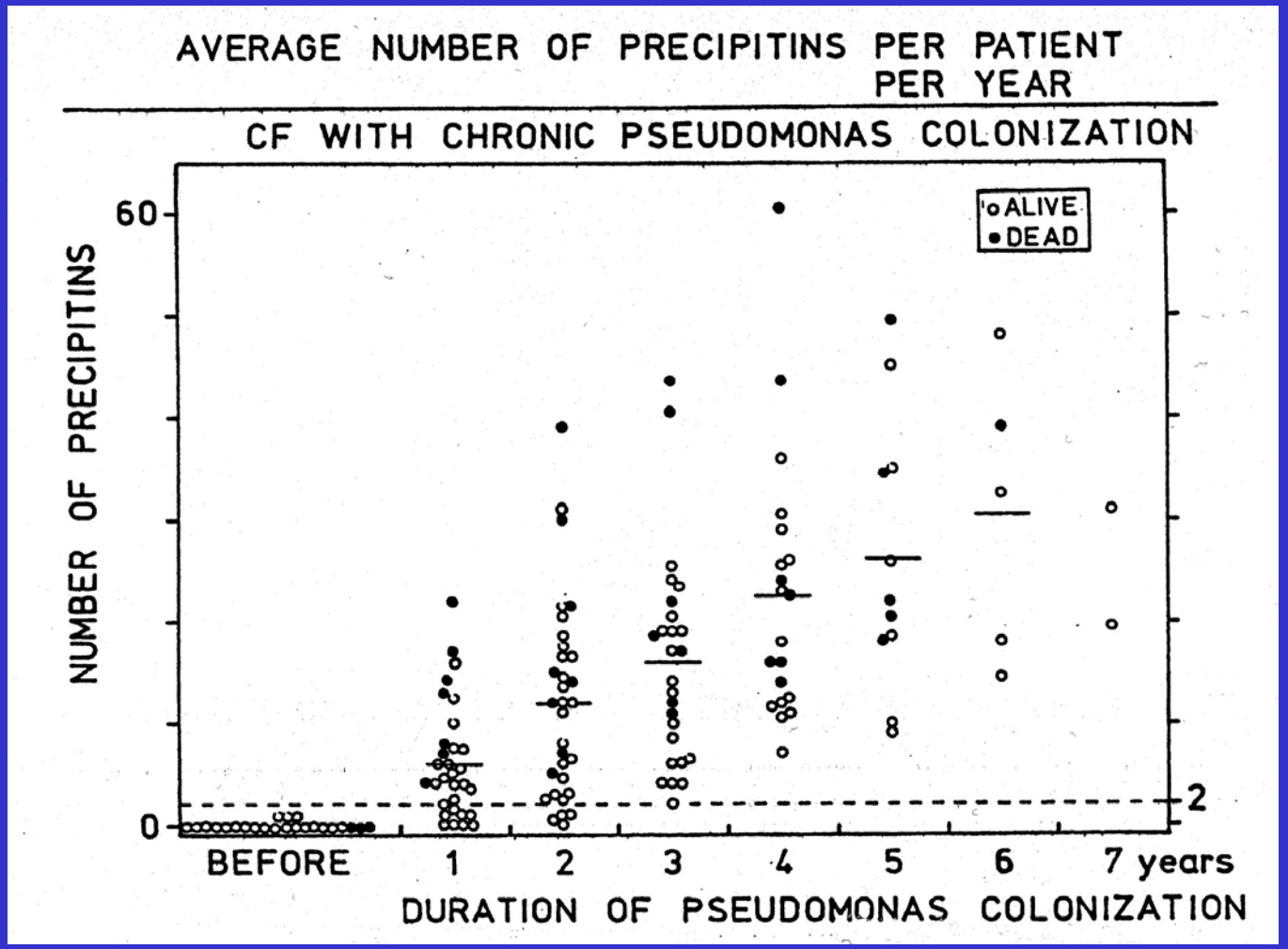
Transcription factor level

”Medleys”

Interesting in an immune modulation setting.

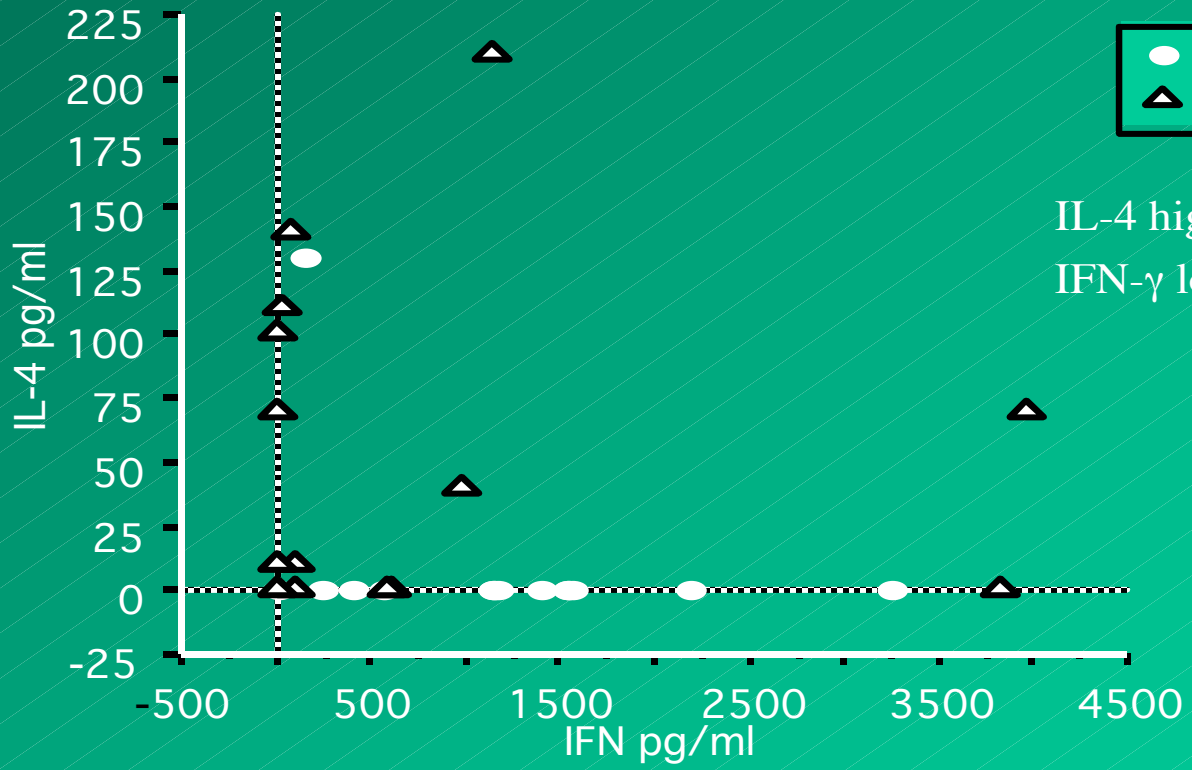
L Zhou et al. Immunity 2009

Previous



Høiby et al. Scand. J. Resp. Dis. 58:65;1977

Dichotomy in CF



IL-4 highest in CF +P ($p<0.005$)

IFN-γ lowest in CF +P ($p<0.05$)

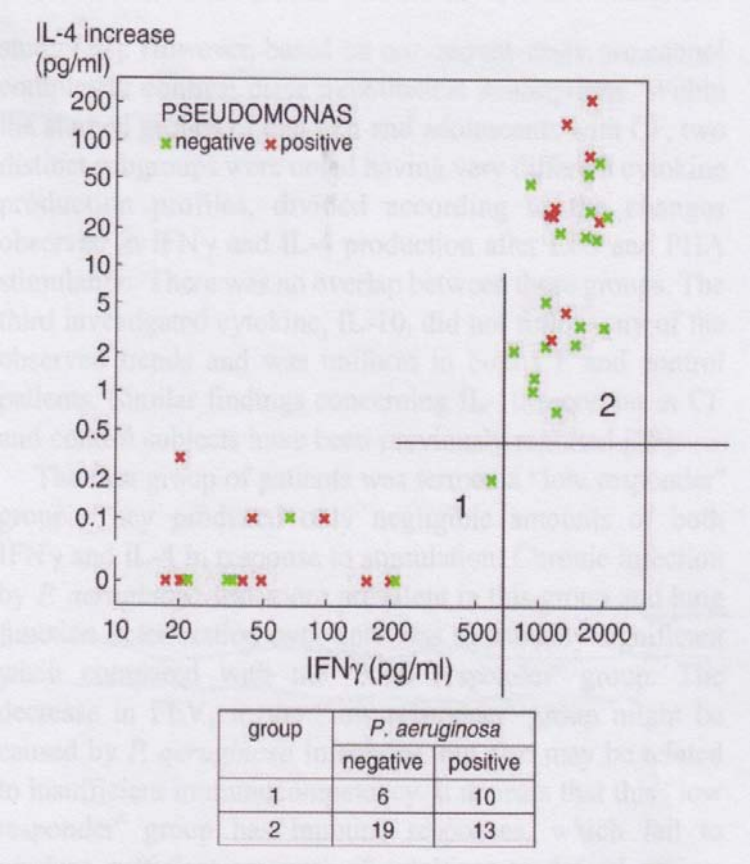
Moser C *et al*. APMIS 2000

Furthermore.

- Decreased IFN-γ RNA expression in CF patients with acute excerbations (Wojnarowski C *et al.* Eur Resp J 1999).
- Decreased IFN-γ secretion by T-cells in CF patients with chronic *P. aeruginosa* lung infection (Moss R *et al.* Clin Exp Immunol 2000).
- CFTR dysfunction in mouse T-cells may be involved in Th2 development (Mueller C *et al.* Am J Respir Cell Mol Biol 2010).
- Th2 response in patients with ABPA (Skov M *et al.* Ped Pulm 1999).

Th1/Th2 dichotomy

Children and young adults



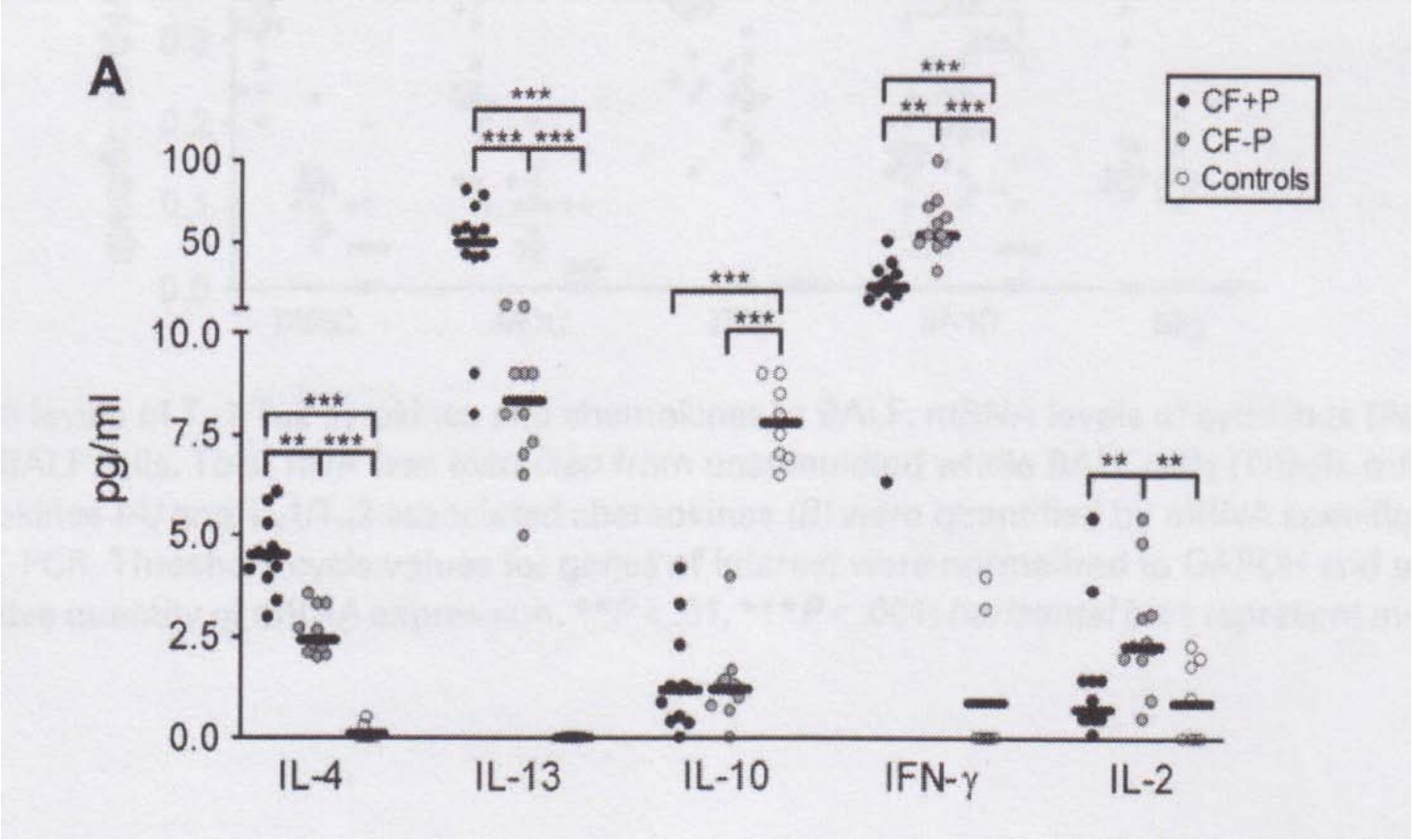
group	*P. aeruginosa*	
	negative	positive
1	6	10
2	19	13

J Brazova et al. 2005.

Non specific stimulation (LPS and PHA).
Long term colonization resulted
in greater IL-4 production ($p<0.02$).

Th1/Th2 dichotomy

Bronchoalveolar lavages



D. Hartl et al. 2005.

Dichotomized response in CF

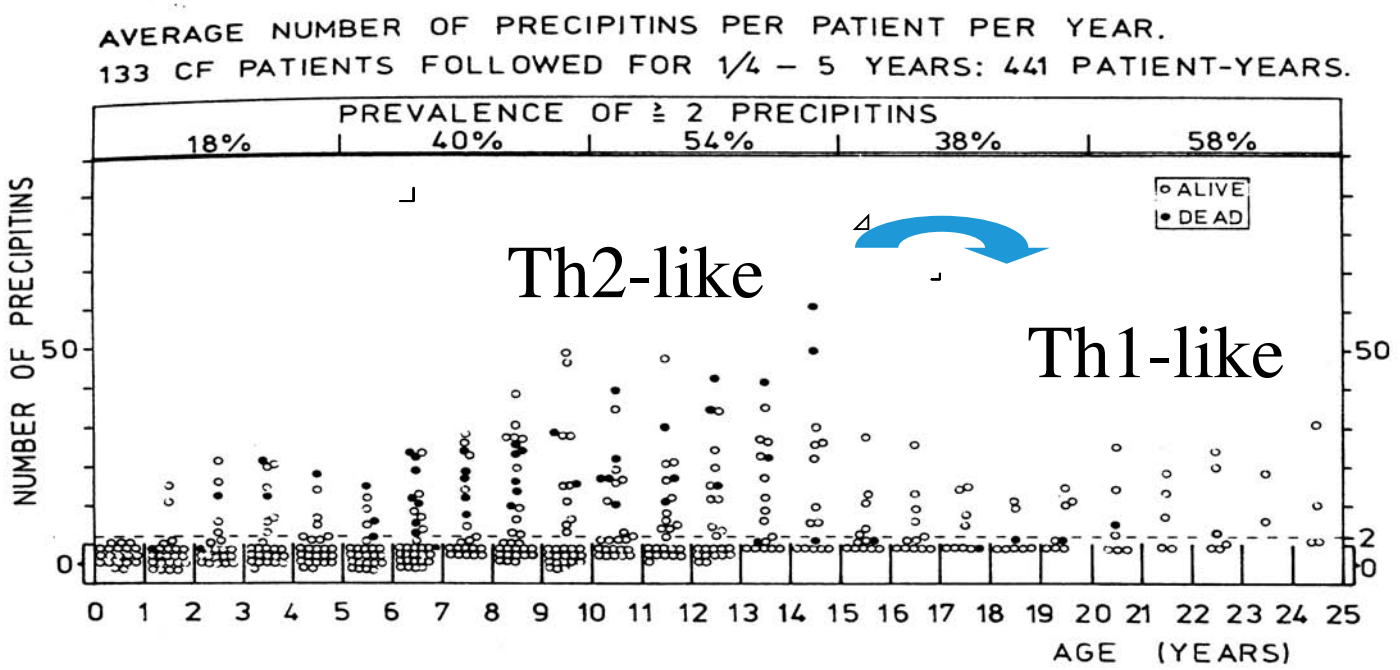
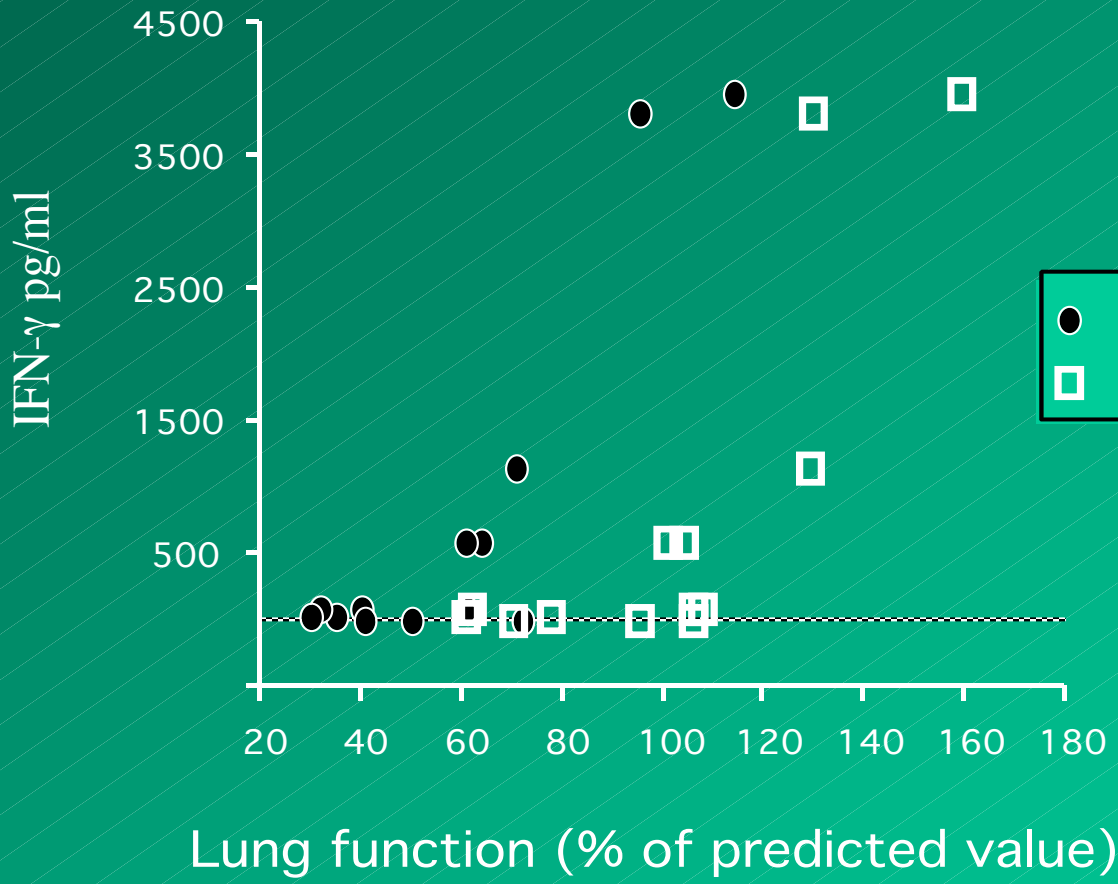


Figure 3. Prevalence and number of precipitins against *P. aeruginosa* in sera from patients with cystic fibrosis classified into different age groups. Each circle represents the average results per year of the crossed immunoelectrophoreses which have been carried out with sera from a given patient. Filled circles represent 18 patients who have later succumbed. The broken line (2 precipitins) is the borderline between normal (0–1 precipitin) and abnormal number of precipitins.

Høiby N, *et al* (Scand J Resp Dis 1977).

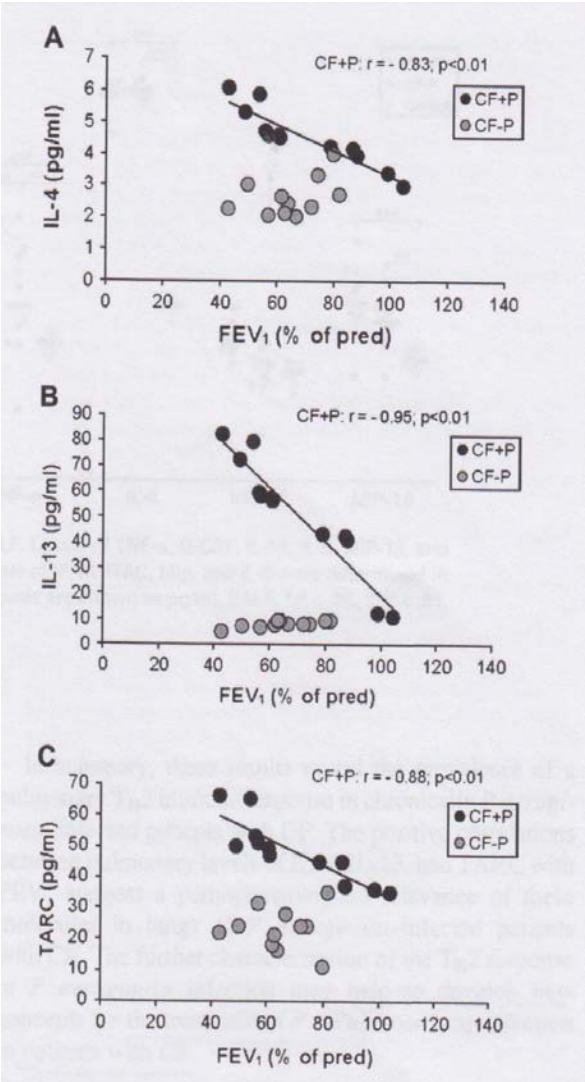
Cytokines and lung function



Moser C *et al*. APMIS 2000

Th1/Th2 and lung function

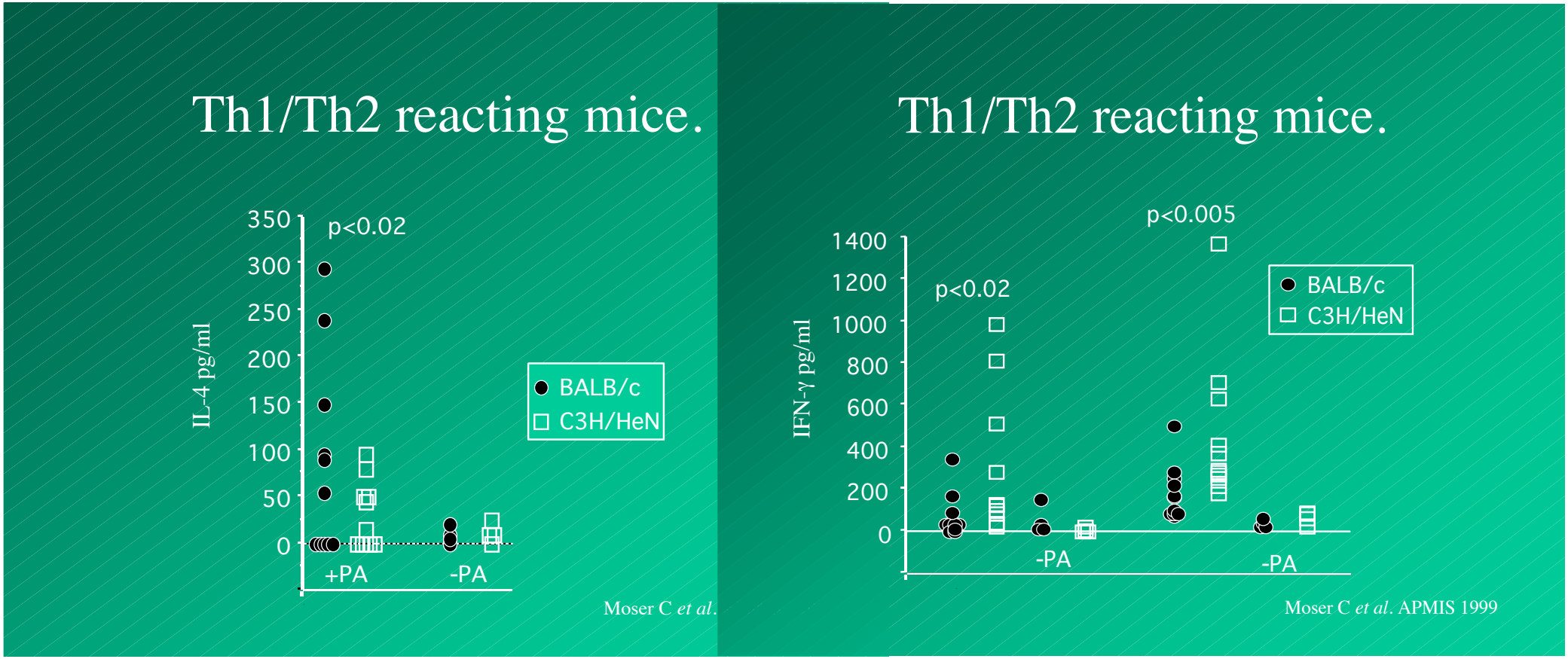
Broncho alveolar lavage



D. Hartl et al. 2005.

Th1/Th2 reacting mice

Chronic *P. aeruginosa* more severe in BALB/c compared to C3H/HeN mice (Moser C *et al*. APMIS 1997)



IFN-γ treatment of rats with chronic *P. aeruginosa* resulted in milder inflammation (Johansen HK *et al*. Clin Exp Immunol 1996)

Involvement of Th17 in CF

Sputum form adult CF patients without exacerbations

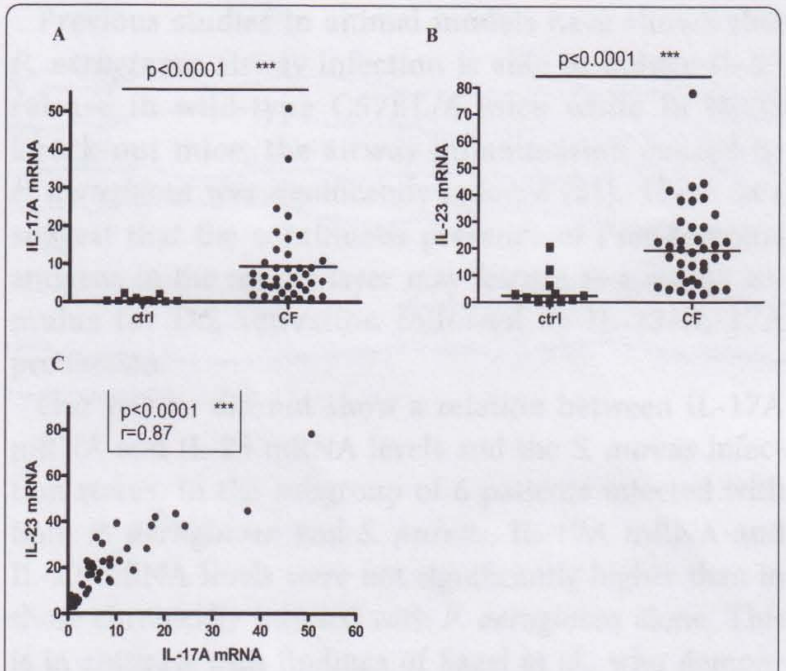


Figure 1 IL-17A and IL-23 mRNA levels: CF group compared to controls. IL-17A **(A)** and IL-23 **(B)** mRNA levels in controls (n = 11) compared to CF group (n = 38). mRNA levels were measured by RT-PCR. Values were normalized to 18S rRNA (ratio multiplied by 10^4). Comparison of controls and CF group was done by nonparametric Mann-Whitney U test ($p < 0.05$ significant). Median levels are shown by the line. **(C)**: Correlation between IL-17A and IL-23 mRNA in the CF group (n = 38). Spearman correlation test was used ($p < 0.05$ significant) (r = correlation coefficient).

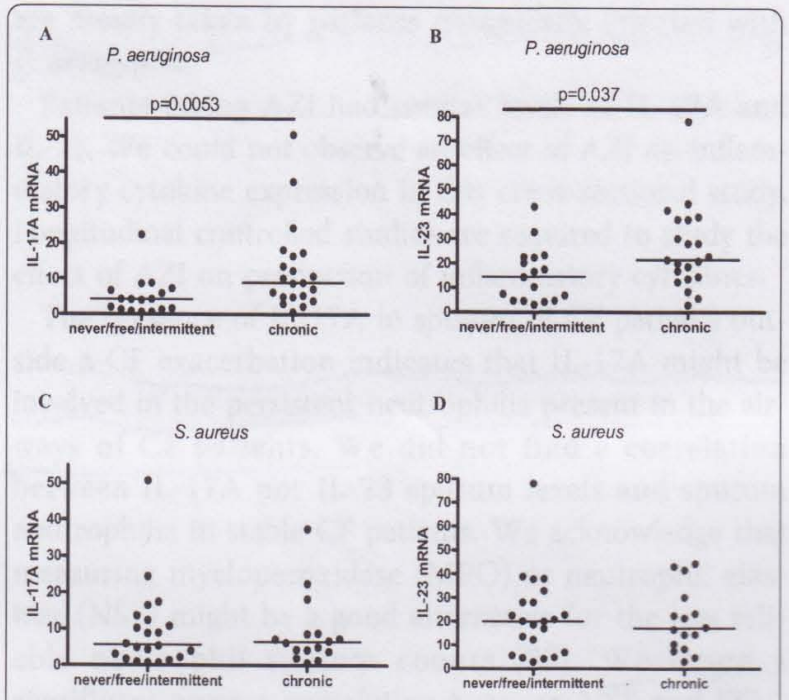


Figure 3 Comparison of IL-17A and IL-23 levels between patients with different colonisation status of *P. aeruginosa* and *S. aureus*. Comparison of IL-17A and IL-23 mRNA levels between patients with different colonisation state of *P. aeruginosa* (A and B) (never/free/intermittent: n = 17 and chronic: n = 21) and *S. aureus* (never/free/intermittent: n = 22 and chronic: n = 16) (C and D) according to the Leeds criteria. mRNA values were normalized to 18S rRNA (ratio multiplied by 10^4). Comparison between the groups was done by nonparametric Mann-Whitney U test ($p < 0.05$ significant). Median levels are shown by the line.

Th17 induced by IL-23.
Characterized by IL-17A
and sometimes IL-22 production.

Induces G-CSF and IL-8 secretion.

A Decraene et al. Resp Res 2010.

Why skewed immune response?

Mobilization of type2 dendritic cells by high dose G-CSF.

Antigen uptake. Mature, migrate and present.

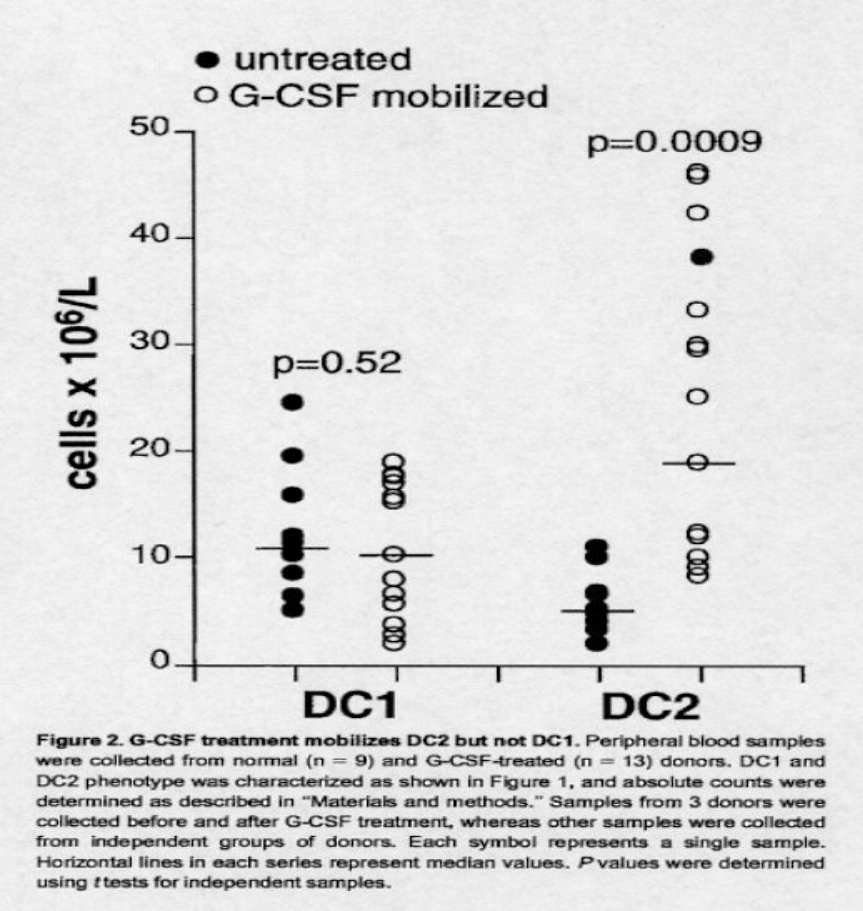


Figure 2. G-CSF treatment mobilizes DC2 but not DC1. Peripheral blood samples were collected from normal (n = 9) and G-CSF-treated (n = 13) donors. DC1 and DC2 phenotype was characterized as shown in Figure 1, and absolute counts were determined as described in "Materials and methods." Samples from 3 donors were collected before and after G-CSF treatment, whereas other samples were collected from independent groups of donors. Each symbol represents a single sample. Horizontal lines in each series represent median values. *P* values were determined using *t* tests for independent samples.

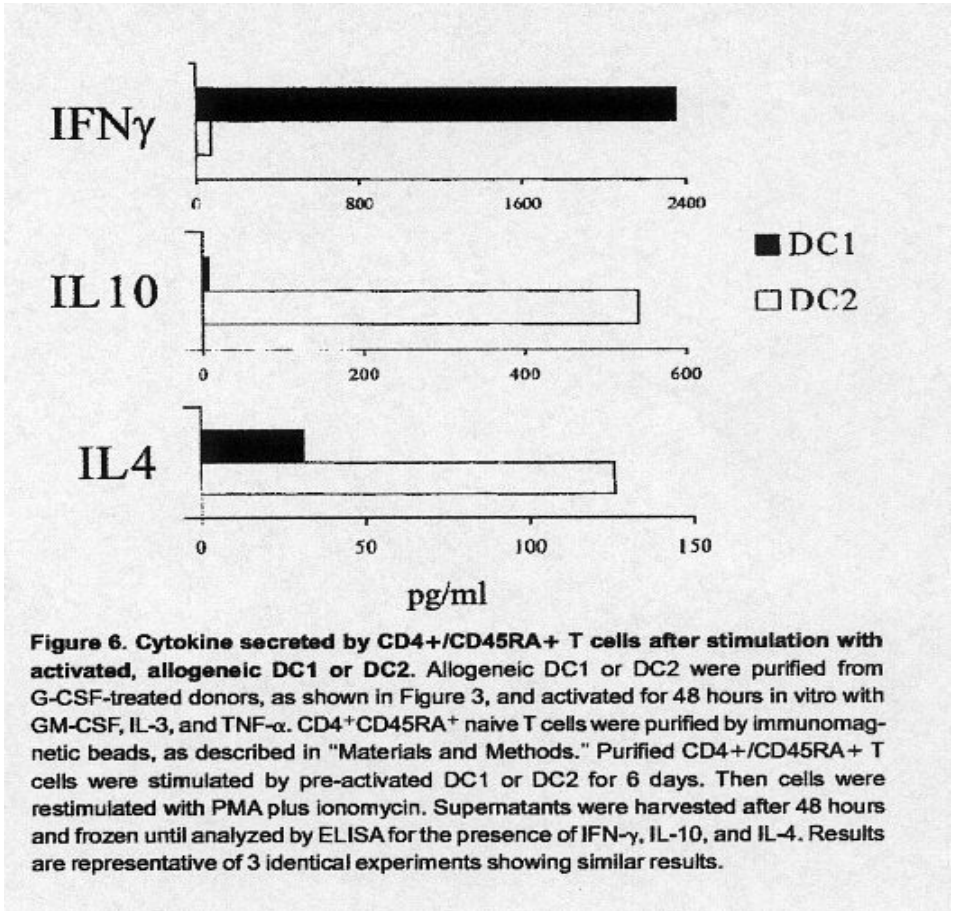
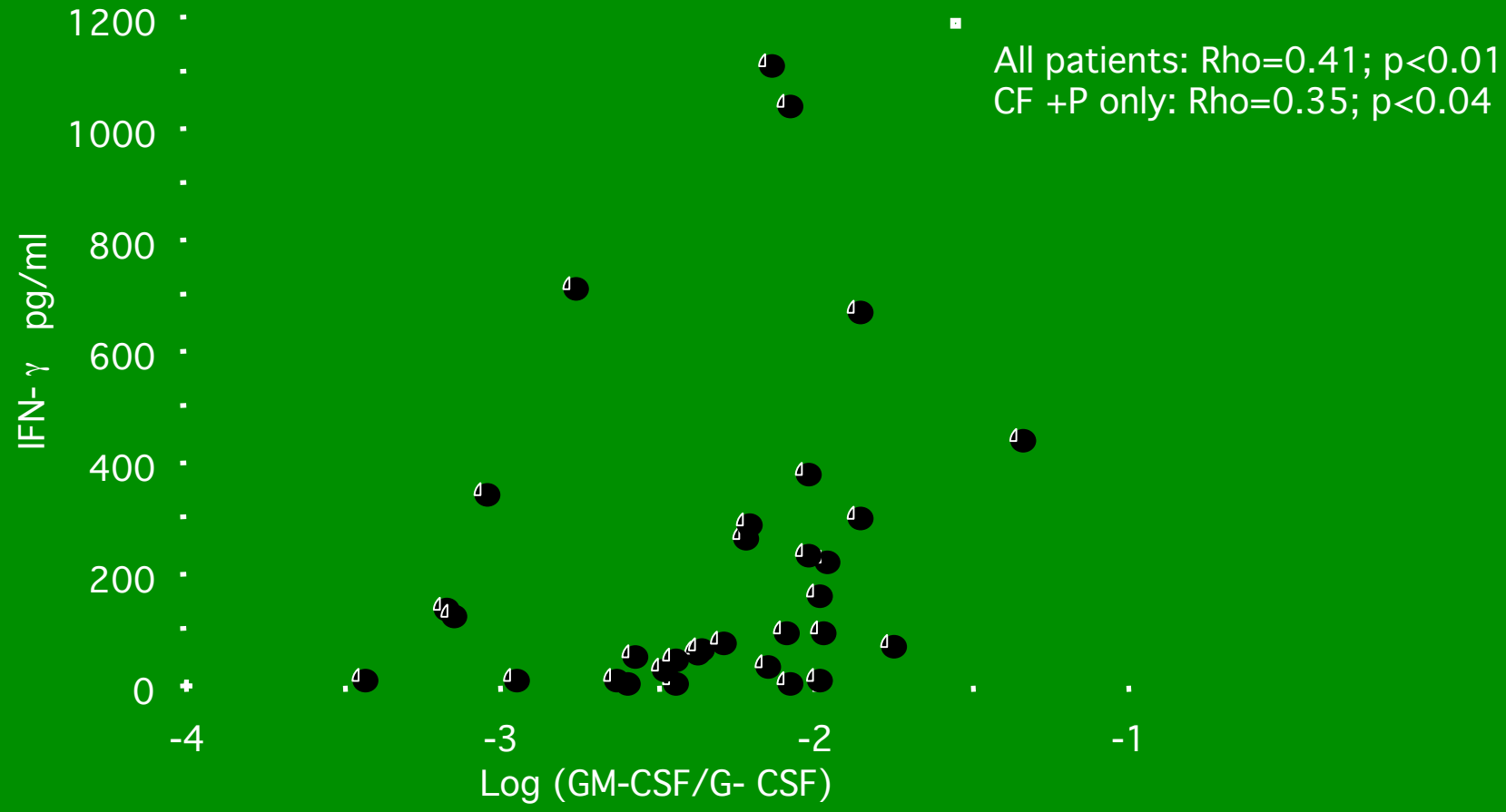


Figure 6. Cytokine secreted by CD4+/CD45RA+ T cells after stimulation with activated, allogeneic DC1 or DC2. Allogeneic DC1 or DC2 were purified from G-CSF-treated donors, as shown in Figure 3, and activated for 48 hours in vitro with GM-CSF, IL-3, and TNF-α. $CD4^+CD45RA^+$ naive T cells were purified by immunomagnetic beads, as described in "Materials and Methods." Purified CD4+/CD45RA+ T cells were stimulated by pre-activated DC1 or DC2 for 6 days. Then cells were restimulated with PMA plus ionomycin. Supernatants were harvested after 48 hours and frozen until analyzed by ELISA for the presence of IFN-γ, IL-10, and IL-4. Results are representative of 3 identical experiments showing similar results.

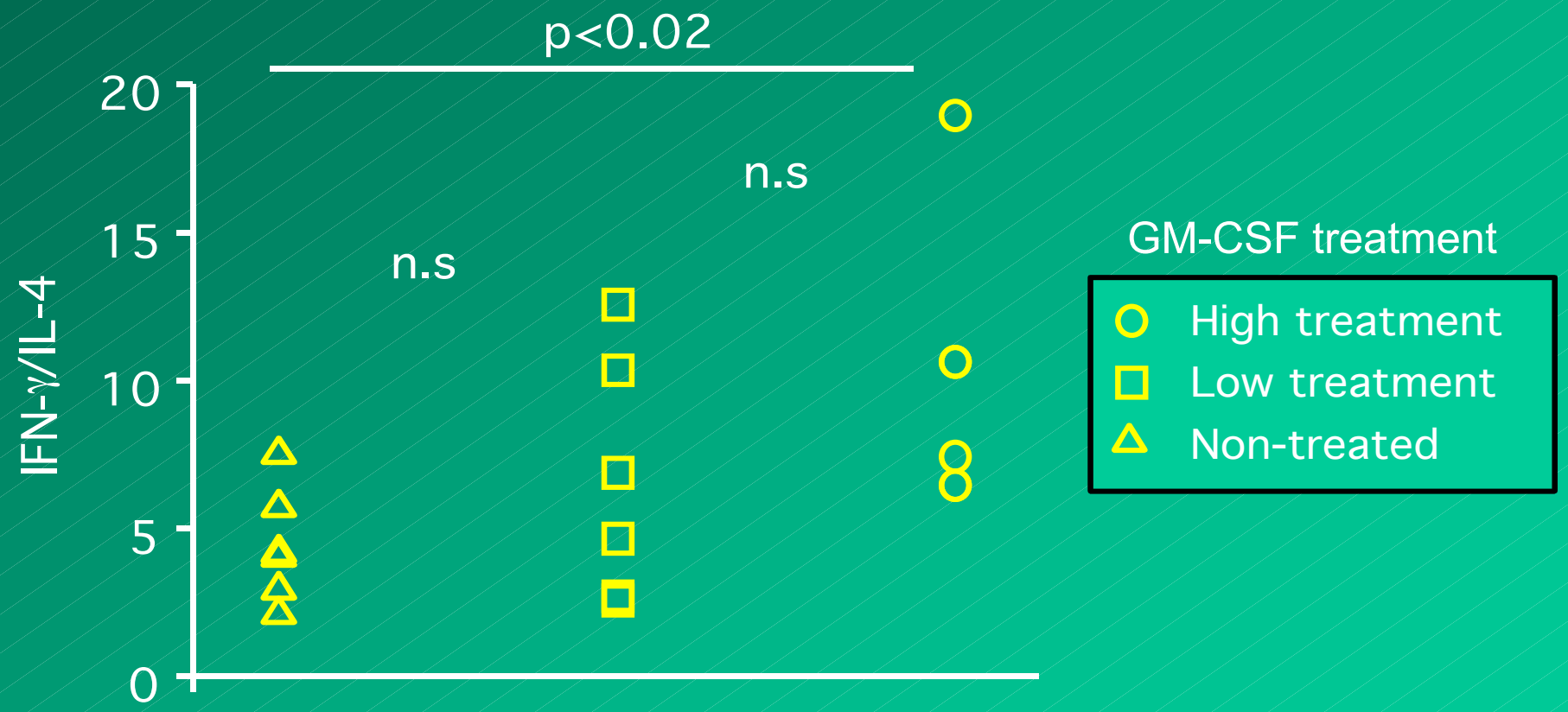
Arpinati M, *et al*. Blood, 2000.

GM-CSF/G-CSF and CF
Th1/Th2



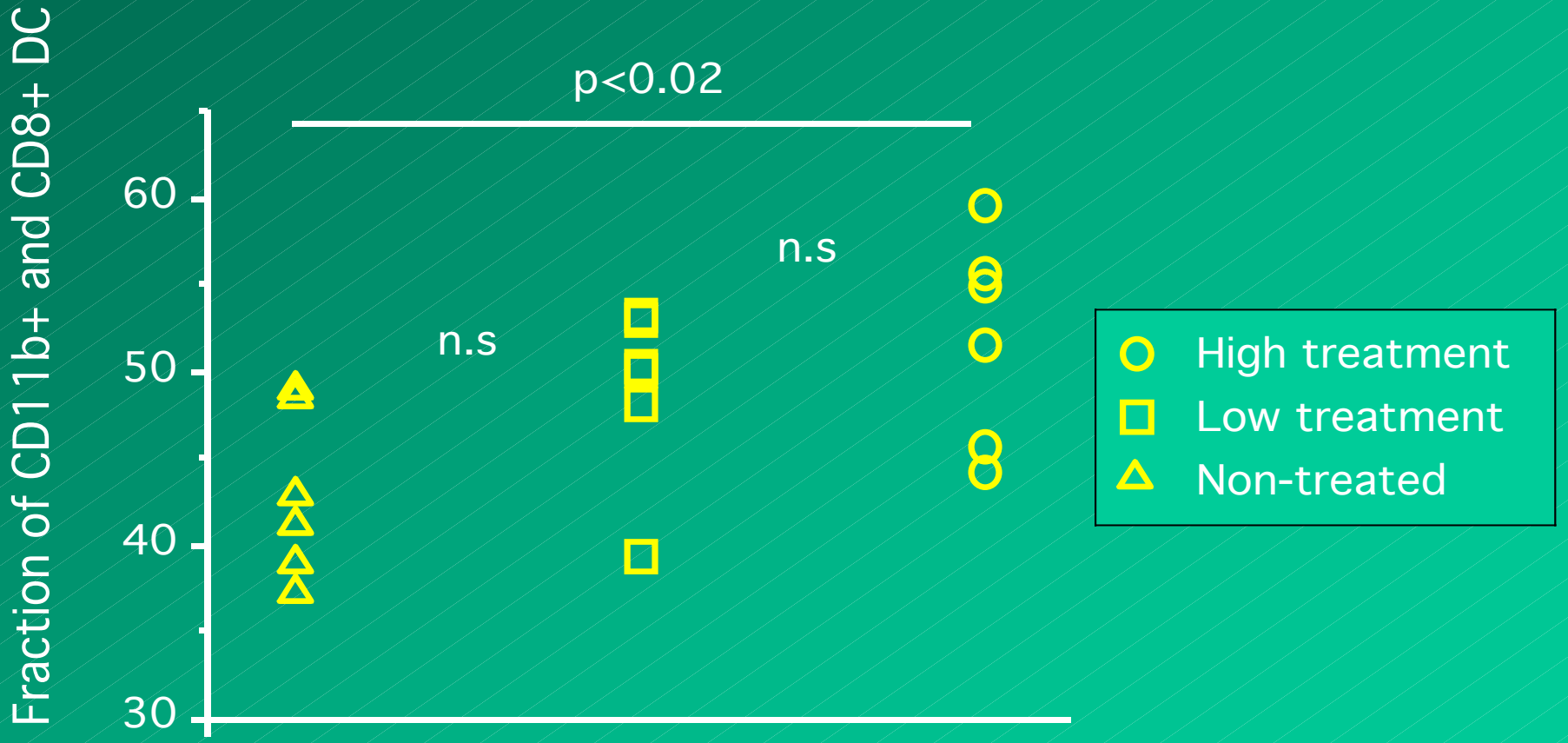
Moser C *et al*. APMIS 2005

Pulmonary IFN-γ/IL-4 ratio



Moser *et al* 2002

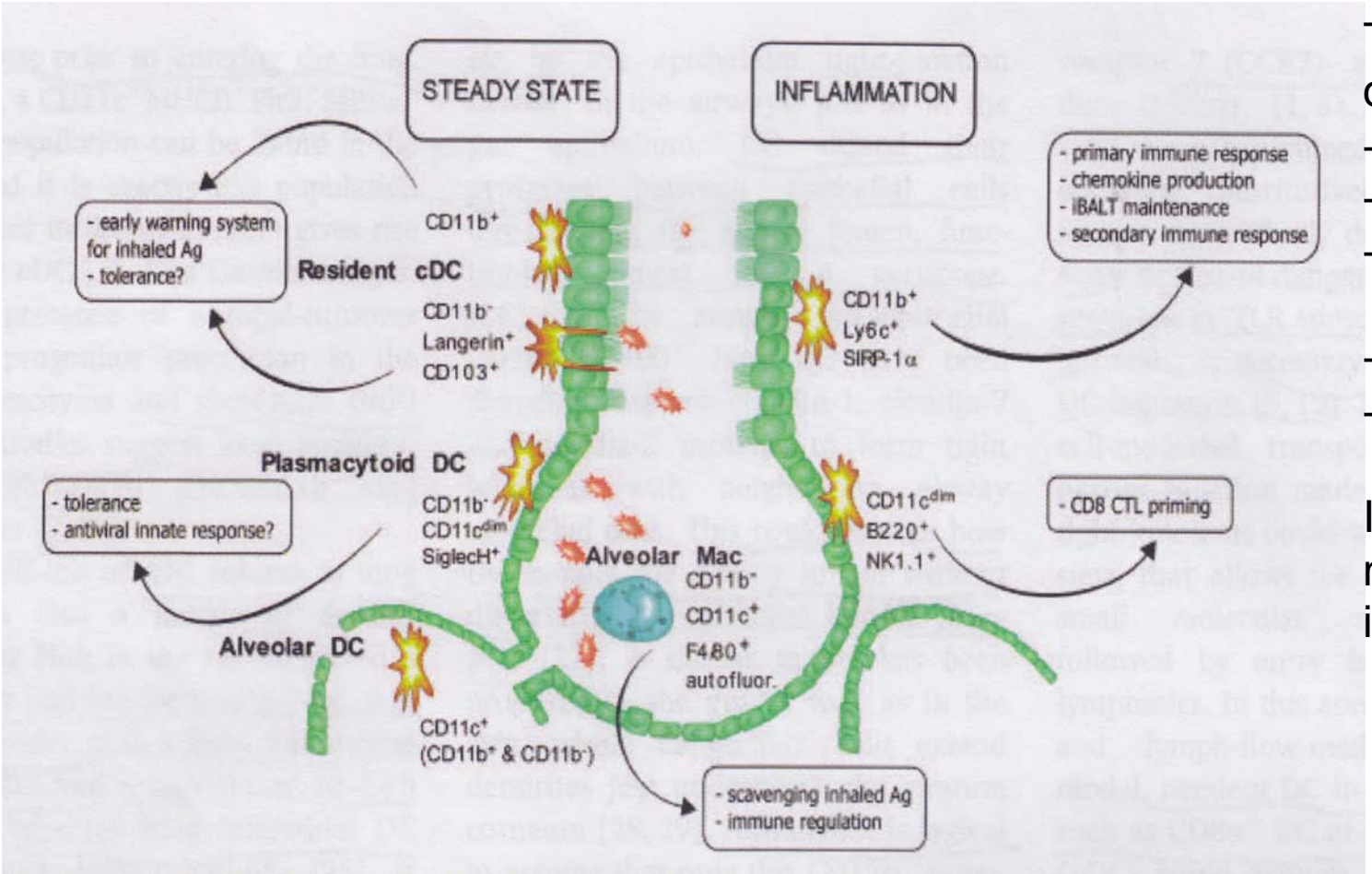
Fraction of double positive DCs (CD11b and CD8α)



Moser *et al*.

Pulmonary DCs much more complex.

Five distinct lung DC subsets based on **surface markers and location.**



Type of generated response dependent on:
-Cytokine environment
-Danger signals
-Pathogen recognizing recoptors (PRRs)
-Pathogen
-Antigen processing

If harmless antigen with minor additional signals induce Treg.

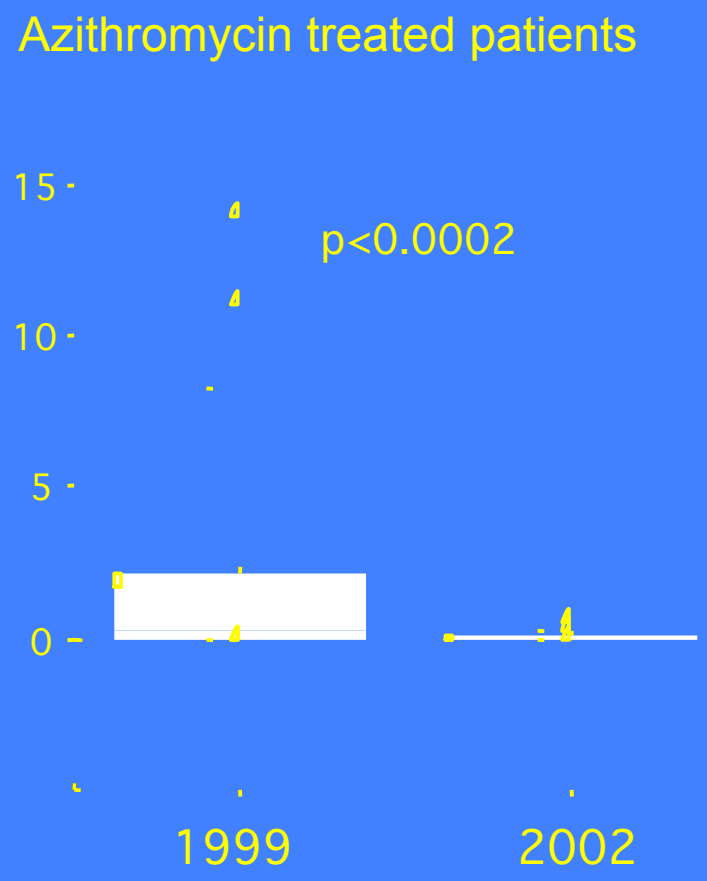
M Plantinga, *et al*. Eur J Immunol, 2010.

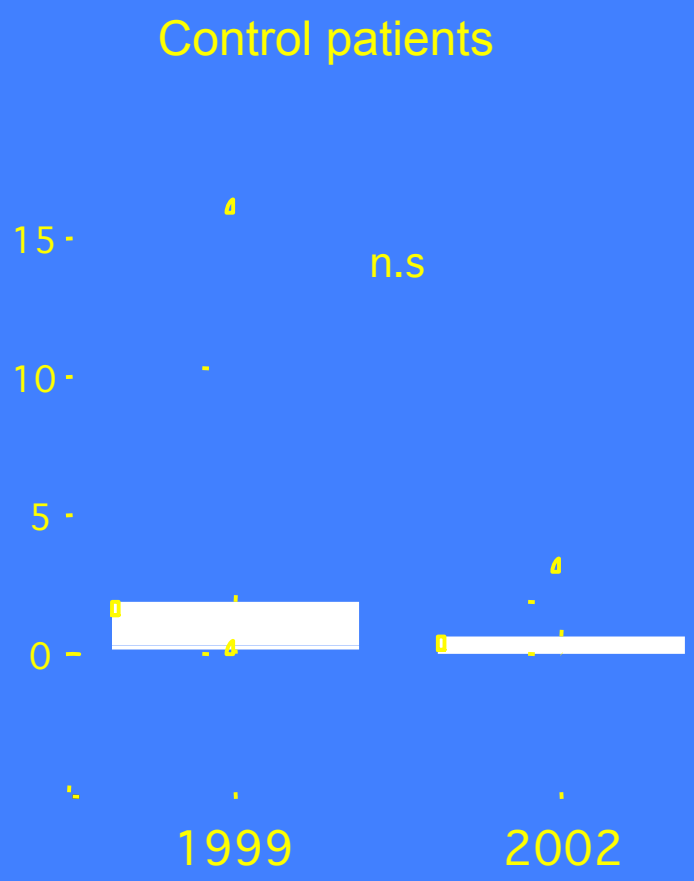
Pulmonary Natural Killer cells

Mouse-strain	**C3H/HeN (Th1)**			**BALB/c (Th2)**		
Day	**0**	**1**	**2**	**0**	**1**	**2**
IFN-γ (pg/ml)	448	568	1755	59	934	22
GM-CSF (pg/ml)	13	46	101	17	20	23

H. Calum, C. Moser *et al*. APMIS 2003.

Azithromycin: IL-4/IFN-γ ratio





Moser *et al.*

Immune modulation in CF.

- Difficult and complex.
- Hippocratic oath (original version) ”….. and never do harm to anyone”.
- Delicate balance (except for ABPA and other allergies).
- What to expect and when - Low degree of lung tissue loss.
- Target/administration – lymph nodes and/or lung tissue.
- Direct or indirect (microbial modulation).

The Th1/Th2 dichotomy in chronic pulmonary *Pseudomonas aeruginosa* biofilm infection.

Conductive airways
Limited immune recognition and limited immune response
=> Limited tissue damage

Trachea

IFN-γ
Stimulation of cellular immunity
Activation of Mϕ

Th_{17} T_{reg} ?

Th_1

More Th_1dominated response
=>Reduced immune complex disease
=>Increased phagocytosis of apoptotic PMNs
=>Reduction in inflammation
=>Reduced tissue damage

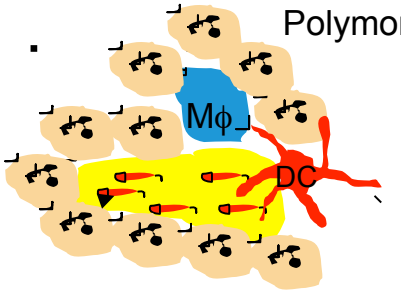
Induction of G-CSF and IL-8
=>Increased PMN mobilization and recruitment
=>Increased tissue damage
=>Induction of Th2?

Th_2

IL-4, IL-5, IL-9 and IL-13
Stimulation of humoral immunity
(Activation of mastcells and eosinophils)
=> immune complex disease
=> persistent activation of PMNs
=> collateral damage

Respiratory airways
Compromised sterility
Immune recognition and immune response
⇒Recruitment of inflammatory cells
=> Tissue damage

Alveolar macrophages (Mϕ) initiate innate immune response
IL-1b, IL-6, TNF-a, IL-8, G-CSF
Polymorphonuclear neutrophils



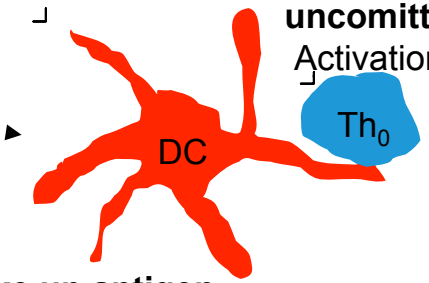
Pseudomonas aeruginosa biofilm

Alveole

NK

Immature dendritic cells (DC) take up antigen
Mature and migrate to secondary lymphoid tissue

Mature dendritic cells present antigens to uncomitted T-helper cells
Activation of adaptive immune response



Th_0

Role of DC subtypes?

Modified from Jensen, Givskov, Bjarnsholt and Moser. FEMS 20

Summary and conclusion

- Adaptive immune response contributes to pathophysiology in chronically infected CF patients by Th2 skewing and Th17 responses without IL-22.
- Role of Treg cells interesting and have to be further clarified.
- Maybe future adjunctive treatment target. Several possible strategies.
- Effect of immune modulatory treatment difficult to appreciate and may not be evident until after long term treatment.

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